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Investigating the Domains' Motions of an Asymmetric ABC Transporter
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ATP-binding cassette (ABC) transporters constitute one of the largest transmembrane protein families found in all kingdoms of life. These ATP-driven machineries are essential for many physiological processes by regulating the transport of lipids, sterols, peptides, toxins, nutrients, drugs and ions across membranes. The common ABC structural core consists of two transmembrane domains (TMDs), and two nucleotide-binding domains (NBDs), which provide two ATP-binding sites. The TMDs are assembled to form the substrate translocation pathway, switching from an inward-facing to an outward-facing conformation. These motions are tightly coupled with those of the NBDs, where ATP-binding and -hydrolysis induce dimerization and dissociation, respectively. Asymmetric ABC transporters feature specific mutations in one of the two ATP-binding sites (the degenerate site), resulting in a reduced ATPase activity compared to the non-mutated site (the consensus site). It has been proposed that in asymmetric ABC transporters ATP remains bound at the degenerate site, while ATP-binding and -hydrolysis at the consensus site drive the transport cycle.
Here, we investigate the effects of ATP-binding and domains' motions of the heterodimeric transporter TM287/288 (Hohl et al., Nat. Struct. Mol. Biol. 2012). We have performed molecular dynamics (MD) simulations, for a total simulation time of ca. 10 microseconds, on the structure of TM287/288 in different nucleotide-bound states. Our results show that in the absence of ATP at the consensus site, the simulation systems with and without ATP at the degenerate site do not completely dissociate at the NBD level, but reach an intermediate state in which contacts between the NBDs are still observed. However, the pattern of interactions at the NBD interface depends on the presence or the absence of the nucleotide at the degenerate site.

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Multiple Membrane-Compatible Conformations of an Substrate-Binding Component of ECF Transporters
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Energy-coupling factor (ECF) transporters compose of a unique subfamily of the vast ATP-binding cassette (ABC) transporters. Unlike other ABC transporters, the basic architecture of ECF transporters is not symmetric, that their membrane sections include two structurally unrelated units: an energy-coupling component EcfT and a substrate-binding component EcfS. The distinctive composition of ECF transporters is likely to associate with a completely novel transport mechanism. Although several crystal structures of different isolated EcfS have been resolved, the structure of a full ECF transporter complex was available only very recently. Surprisingly, the EcfS orientation in the full ECF transporter is almost perpendicular to an ideal orientation should the EcfS exist as an isolated transmembrane protein. Based on the crystal structures, it is proposed that the transport mechanism of ECF transporters involves the reorientation of EcfS in membrane. Starting with the crystal structure of the folate ECF transporter, we have performed a series of MD simulations to evaluate the stability of EcfS with different orientations in membrane, either isolated or within the full ECF transporter. It is found that the substrate-free EcfS is able to maintain the membrane-parallel orientation regardless of the EcfT binding. That is, in addition to the ideal orientation for an isolated EcfS, the membrane-parallel orientation can be stabilized by the partition of hydrophilic residues near the membrane surface, as well as the accommodation of several charged residues near the membrane center by an enclosed lipid head group in the putative substrate binding site. The tilt angles of the two identified stable orientations of EcfS differ by ~50 degrees, and the membrane-parallel form shows slightly higher structural deviations due to repartitioned N- and C-terminal helices near the membrane surface.

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EPR Spectroscopy of $MOLB_2C_2$-A Reveals Mechanism of Transport for A Type II Molybdate Importer
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ABC importers are embedded in the plasma membrane of all bacteria. There they facilitate the uptake of several vital nutrients and cofactors. Due to differences in structure and activity, ABC importers are divided into two types. Type I importers have been well studied, but most of what we know about Type II importers has been observed in studies of the vitamin B12 importer, $BtuC_2D_2$. $MolB_2C_2$ (formally known as HI1470/71) is also a Type II importer, but its substrate, molybdate, is about 10x smaller than vitamin B12. To understand mechanistic differences among Type II importers, we focused our studies on MolBC where alternative conformations may be required to transport its relatively small substrate. To investigate the mechanism of MolBC, we employed electron paramagnetic resonance (EPR) spectroscopy with the transporter imbedded in detergent micelles and liposomes (spherical lipid bilayers). The use of both detergent and lipid have allowed us to identify conformational changes in MolBC and the impact of the lipid environment. We observed that nucleotide binding and hydrolysis shift the conformation of MolBC at both the cytoplasmic and periplasmic gates. Mechanistic differences between MolBC and other Type II transporters highlight potential effects of substrate size on transport. Combining our results, we propose a mechanism for MolBC-A embedded in a lipid bilayer, which gives new insight in the transport of small substrates.

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Combining in Vitro with in Silico Studies to Obtain Insights into Substrate Releasing State of the Multidrug Resistance Protein P-Glycoprotein
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The human genome contains 48 ATP-Binding Cassette (ABC) proteins. We focus on the multidrug resistance transporter P-glycoprotein that transports an extraordinarily diverse range of structurally unrelated drugs, xenobiotics and endogenous substrates. P-glycoprotein is expressed at barrier tissues including the blood-brain-barrier, the intestine, kidney, liver and macrophages. Cancer cells acquire resistance to chemotherapy when expressing P-glycoprotein. It is well established that ATP binding and its subsequent hydrolysis drives the transport process in all ABC transporters. In contrast, most details regarding the mechanism of substrate recognition, uptake and binding to P-glycoprotein, and the mechanism by which substrate binding in the transmembrane domains is coupled to ATP hydrolysis in the nucleotide binding domains remain unknown.
The bacterial homologue of P-gp, Sav1866 (Staphylococcus aureus), was the first ABC exporter crystallized. The same fold was later observed in other transporters of the ABCB family, suggesting a conserved architecture across the ABCB exporter family. Although ABC exporters have now been crystallized in several conformations, uncertainty remains regarding the physiological conformation of these structures. None of the crystal structures is fully compatible with all biochemical evidence.
We combined modeling with experiments to address these issues. Homology modeling and MD simulations were used to determine the equilibrium conformation of ATP-bound P-glycoprotein in a membrane environment. In contrast to the conformations observed in crystal structures, the wing shape structure is unstable in the membrane environment. The conformation observed by MD simulations is devoid of the wing-shape, but in agreement with the bulk of the biochemical data.
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Refined Structures of Mouse P-Glycoprotein
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The recently determined C. elegans P-glycoprotein (Pgp) structure revealed significant deviations compared to the original mouse Pgp structure, which suggested possible misinterpretations in the latter model. To address this concern, we generated an experimental electron density map from single-wavelength anomalous dispersion phasing of an original mouse Pgp dataset to 3.8 Å resolution. The map was significantly improved in detail compared to the original MAD map and revealed several regions of the structure that required de novo model building. The improved drug-free structure was refined to 3.8 Å resolution with a 9.4% and 8.1% decrease in Rwork and Rfree, respectively (Rwork=21.2%, Rfree=26.6%) and a significant improvement in protein geometry. The improved mouse Pgp model contains ~ 95% of residues in the favorable Ramachandran region compared to only 57% for the original model. The registry of six transmembrane helices was adjusted, revealing amino acid residues involved in drug binding that were previously unrecognized. The greatest changes required registry shifts (rotations and translations) for TM4 and TM5 as well as the addition of three N-terminal residues to the model - each validated with new mercury labeling and anomalous fourier density. The corrected position of TM4, which forms the frame of a portal for drug entry, was shifted > 6 Å from its original position. The drug translocation pathway of mouse Pgp is 96% identical to human Pgp and is enriched in aromatic residues that likely play a collective role in allowing a high degree of polyspecificity in substrate recognition.